Intra-articular bupivacaine: A literature review including a pilot study investigating the safety of intra-articular bupivacaine to chondrocytes

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DECLARATION:

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ABSTRACT

Background: Today, intra-articular bupivacaine injections are common practice, with very good analgesic effects. This method of analgesia is utilized by general practitioners, orthopedic surgeons and anesthesiologists. Unfortunately, since 2004, more than 200 cases of chondrolysis were noted following intra-articular bupivacaine infusion. Numerous studies have investigated the safety of intra-articular bupivacaine. The aim of this thesis was to quantify the risk of chondrolysis with intra-articular bupivacaine and to guide medical practitioners in safe practice.

Methods: A literature review was done of the most recently published original research, meta-analysis and review articles. Adverse outcomes were mostly associated with bupivacaine infusions, rather than with single intra-articular doses of bupivacaine. Limited data on the safety of a single dose of intra-articular bupivacaine was found. We conducted a pilot study to investigate the safety of a single dose of intra-articular bupivacaine. A paired, case-controlled, experiment was done using four merino sheep. The nul hypothesis was that a single dose of intra-articular bupivacaine did not cause chondrolysis when compared with a dose of intra-articular normal saline. Our results were added to the thesis to further quantify the risk.

Results: Numerous in vitro and in vivo studies confirmed that bupivacaine is chondrotoxic. The chondrotoxic effect is time, dose and concentration dependent. Our pilot study revealed that single dose intra-articular bupivacaine may also be unsafe for chondrocytes.

Conclusion: The administration of intra-articular bupivacaine is toxic to chondrocytes. The chondrotoxic effect of intra-articular bupivacaine is time, dose and concentration dependent. Infusions of intra-articular bupivacaine should be contra-indicated. From the limited data available on single dose intra-articular bupivacaine, it appears that it is also chondrotoxic. Magnesium, morphine and clonidine may be safer for intra-articular use, but this needs further investigation.

OPSOMMING

Agtergrond: Vandag word bupivacaine gebruik as intra-artikulêre inspuiting met baie goeie analgetiese effek. Hierdie metode word ontgin deur, onder andere, algemene praktisyns, ortopediese chirurge en anestesioloë. Sedert 2004 is meer as 200 gevalle van chondrolise aangeteken na die toediening van intra-artikulêre bupivacaine infusies. Talle studies het gevolg om die veiligheid van intra-artikulêre bupivacaine te ondersoek. Die doel van hierdie skripsie is om die risiko verbonde aan intra-artikulêre bupivacaine te kwantifiseer en aan mediese praktisyns veilige riglyne te stel.

Metode: ‘n Literatuur oorsig is gedoen, insluitende die mees onlangs gepubliseerde oorspronklike navorsing, meta-analises asook oorsig artikels. Ongunstigde uitkomstes is meestal geassosieer met intra-artikulêre bupivacaine infusies, eerder as met enkel dosering intra-artikulêre bupivacaine. Beperkte data met betrekking tot die gebruik van bupivacaine as enkel dosering intra-artikulêr is beskikbaar. Om die veiligheid van enkel dosering intra-artikulêre bupivacaine te ondersoek is ‘n loodsstudie gedoen. Dit is ‘n gevalle-kontrole in-vivo eksperiment, met vier merino skape. Die nul hipotese is dat ‘n enkel dosering intra-artikulêre bupivacaine nie meer chondrolise veroorsaak, as ‘n enkel dosering intra-artikulêre normale saline nie. Die resultate is ingesluit as deel van die stawende data om die risiko te kwantifiseer.

Resultate: Talle in-vitro asook in-vivo studies het bevestig dat bupivacaine chondrotoksies is. Die toksiese effek is tyd, dosis en konsentrasie afhanklik. Ons loodsstudie het ook getoon dat enkel dosering intra-artikulêre bupivacaine moontlik skadelik vir kraakbeen kan wees.

Gevolgtrekking: Die toediening van intra-artikulêre bupivacaine is toksies vir chondrosiete. Die chondrotoksiese effek is tyd, dosis en konsentrasie afhanklik. Infusies van intra-artikulêre bupivacaine is dus gekontraïndikeer. Die beperkte data oor enkel dosering intra-artikulêre bupivacaine dui ook moontlik chondrotoksisiteit aan. Magnesium, morfien en clonidine kan moontlik as veiliger alternatief oorweeg word, maar benodig verdere navorsing.

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DEDICATIONS

I dedicate this thesis to my wife, Annemarie, and my two sons, Guillem and Jakob. Thank you for all your love, patience and support over the last four years.

TABLE OF CONTENTS

PREFACE

The aim of this thesis is to review the literature on the effect of intra-articular bupivacaine on chondrocytes. As most of the adverse outcomes were associated with intra-articular bupivacaine infusions, the effect of a single dose of intra-articular bupivacaine on chondrocytes was further investigated by conducting a pilot study on four adult merino sheep. The literature review will be discussed in chapter 1 of this thesis and the results of the pilot study will be disclosed in chapter 2, followed by a discussion of our findings and a conclusion in chapters 3 and 4 respectively. We trust that it will give more insight and guidance into the safe practice of intra-articular bupivacaine.

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CHAPTER 1
LITERATURE REVIEW: INTRA-ARTICULAR BUPIVACAINE

A literature review was done of the available published literature, for the time period of 1985 to 2015.

1.1 INTRODUCTION

Today, intra-articular bupivacaine injections are common practice amongst orthopedic surgeons, general practitioners and anesthetists with very good analgesic effects.[1-2]

This method of pain control is utilized in the outpatient, inpatient and perioperative setting. Bupivacaine has been used either as the sole drug of the injectate, or in combination with additives such as clonidine, opiates, corticosteroids and epinephrine.[3]

This practice of analgesia has however not been without local risks to joints. There are even reports of possible systemic toxicity following intra-articular administration of bupivacaine.[4-5]

According to Meining et al.[6], peak plasma levels after therapeutic intra-articular dosages occur approximately 20 minutes after administration, but the actual plasma level is normally eight to ten times less than the human convulsion threshold. Certain surgical techniques compromising the cartilage and bone structure might increase the risk of systemic uptake.[6] However, the reported incidence of systemic compromise after intra-articular bupivacaine is extremely low.

More prevalent and of greater concern, are reports of a possible chondrotoxic effect of intra-articular bupivacaine, especially in cases of intra-articular infusion.[7-9]

Between 2004 and 2012 more than 200 cases of chondrolysis were noted following intra-articular bupivacaine infusion. The mean age of the patients who developed chondrolysis was only 30 years, suggesting bupivacaine as the possible cause.[11] In a series of 19 patients who received intra-articular infusions of bupivacaine and epinephrine, 12 developed chondrolysis. [10]

Numerous in vitro and in vivo studies have investigated the safety of intra-articular bupivacaine to chondrocytes.

1.2 IN VITRO STUDIES

Drogoo et al.[13] claimed that intra-articular bupivacaine infusions could be utilized with the provision that the infusion duration did not exceed 48 hours. Interestingly, they found that bupivacaine in combination with epinephrine caused significantly more chondrotoxicity than bupivacaine alone. Human chondrocytes, cultured in a bioreactor that mimicked synovial metabolism, were exposed to 1% lidocaine, and 0.25% and 0.5% bupivacaine. Chondrocytes were exposed to these solutions alone and with epinephrine. Cell viability was assessed 24, 48 and 72 hours after continuous infusion. Similar chondrocyte necrosis was found in 0.25% and 0.5% bupivacaine groups at 24 and 48 hours when compared to control groups. When epinephrine was added, significantly greater necrosis was seen ($p < 0.05$) at all intervals. At 72 hours, the 0.5% bupivacaine alone had caused significant necrosis.[13]

Lo et al.[14] confirmed the chondrotoxic effect of 0.25% bupivacaine, 1% lidocaine and 0.5% ropivacaine on bovine cartilage, which had been sampled from the radial-carpal joint. Significantly worse chondrotoxicity was shown with longer periods of exposure of the cartilage to the local anesthetics.[14]

Hennig et al.[15] found intra-articular bupivacaine 0.5% to be toxic to canine cartilage.[15]

Baker et al.[16] compared the effect of different local anesthetics at different concentrations on human chondrocytes. Cells were exposed to levobupivacaine (0.13%, 0.25%, 0.5%), bupivacaine (0.13%, 0.25%, 0.5%), ropivacaine (0.19%, 0.38%, 0.75%), normal saline and 10% magnesium sulphate, for 15 minutes each. After 24 hours, cell viability was found to be significantly decreased in all local anesthetic groups and appeared to be concentration dependent ($p < 0.05$). There was no reduction in chondrocyte viability after exposure to 10% magnesium sulphate.[16]

Wang et al.[17] evaluated chondrocyte viability and chondrocyte function after exposure to bupivacaine. They used an intervertebral disc organ model, which they harvested from 10 week old mice. They waited 3 days before exposing the cells to bupivacaine to compensate for surgically induced inflammatory responses. The cells were exposed to bupivacaine 0.1%, 0.25% and 0.5%, for 60 minutes. The 0.5% bupivacaine group was also exposed for different time periods of 30, 60 or 120 minutes. Cell viability, collagen synthesis and proteoglycan matrix synthesis was evaluated. They showed a dose and time dependent reduction in cell viability. Bupivacaine 0.5% killed 20% of chondrocytes after 60 minutes and up to 70% after 120 minutes (95% CI 53.2 – 70.1%).

Bupivacaine 0.5% reduced the proteoglycan matrix synthesis three-fold and collagen synthesis four-fold. The authors postulate that the reduced synthetic function that was demonstrated may only be a reflection of chondrocyte loss, and not necessarily reflect an inhibition of chondrocyte function by bupivacaine.[17]

Breu et al.[18] attempted to quantify bupivacaine's chondrotoxic effect on human mesenchymal cells, and compared it to equipotent concentrations of mepivacaine and ropivacaine. After 60 minutes of exposure to the local anesthetics, bupivacaine was found to be significantly more chondrotoxic, reducing cell viability by 5% (± 1%) when compared to mepivacaine's 1% (± 0%) reduction. Ropivacaine proved to be the least toxic.[18]

Beyzadeoğlu et al.[19] showed that both bupivacaine and its enantiomer, levobupivacaine, were chondrotoxic to rat cartilage. Levobupivacaine proved to be more toxic.[19]

In contrast, Erden et al.[20] could not illustrate any increase in inflammation in articular and peri-articular surfaces in a rat model after treatment with levobupivacaine. The authors advocated that levobupivacaine might therefore be a safe alternative.[20] A possible cause for their negative results, might be that the samples were only investigated up to 21 days post intervention, and the changes become more prevalent at longer intervals after exposure.[11]

In an experiment done by Gungor et al.[21], no statistical difference in chondrocyte apoptosis could be demonstrated between bupivacaine and levobupivacaine.[21]

Most of the in vitro studies were not done on full thickness cartilage and therefore might falsely increase the likelihood of positive results. It is also problematic to make assumptions based on in vitro cell cultures. The complexity of the synovial environment, extracellular collagen and proteoglycan matrix interactions can only be mimicked to a certain degree, before compromising the true milieu. In vivo studies have therefore also been done.

1.3 IN VIVO STUDIES

Gomoll et al.[12] divided 30 male New Zealand white rabbits into three groups, to receive either normal saline, bupivacaine 0.25% or bupivacaine 0.25% with epinephrine (1:200 000), via continuous infusion into their glenohumoral joints for 48 hours at a rate of 210μL/h. This dosage was proportional to the normal infusion rate of 4ml/h administered to adult human patients.

Cartilage samples from the glenohumeral joints were taken after one week and chondrocyte viability was evaluated by measuring metabolic sulfate uptake. Sulfate uptake was decreased by 16%, 58% and 53% in the different groups respectively when compared to their control shoulders. The bupivacaine and bupivacaine with epinephrine groups had 50 % ($p = 0.02$) and 56% ($p = 0.009$) greater reduction in sulfate uptake when compared to normal saline. The addition of epinephrine did not cause a statistical difference between the two bupivacaine groups. The authors therefore cautioned against the use of continuous infusion of bupivacaine into joints.[12]

Three years later, in 2009, Gomoll et al.[22] performed another in vivo study on 36 rabbits. They were randomly infused for 48 hours into their glenohumoral joints with either normal saline, bupivacaine (0.25%) or bupivacaine (0.25%) with epinephrine. After 3 months, chondrocyte viability was assessed by measuring proteoglycan synthesis and conventional histology. There were no significant differences amongst the groups in cell count, cell viability and histology results. Gomoll claims that an additional noxious stimulus might be necessary for permanent damage.[22] The critique to this study, based on the work of Drogoo et al.[13], would be that infusions were not extended beyond 48 hours.[13]

After all the alarming results of the studies with local anesthetic infusions, the question of the safety of a single dose of intra-articular bupivacaine was raised. Chu et al.[23] performed an in vivo study on 48 rats, that had 0.9% saline injected into their stifle joints, and either 0.5% bupivacaine or 0.6% mono-iodoacetate injected into their contralateral stifle joints. The mono-iodoacetate served as a positive control due to its known chondrotoxicity. Histological analyses of the chondrocytes were done at one week, four weeks, twelve weeks and six month intervals. Cell viability and cell density were quantified and the histology was interpreted with the Modified Mankin Score (see Table 2.1). A 50% reduction in chondrocyte density was found in the bupivacaine group when compared to its contralateral saline group ($p < 0.01$) at six months. They also showed that acute loss of 75% of viable chondrocytes resulted in severe progressive arthritis after 6 months. It was more prominent after longer intervals since administration.[23]

This delayed arthritic effect seen in the rats compared well with human case studies, in whom symptoms were noticed between 2 to 8 months after exposure to bupivacaine. A possible reason for this delayed onset of arthritis was postulated by Matsen et al.[11], who suggested that an initial reduction of viable chondrocytes later resulted in poorer maintenance of matrix integrity. Alterations in mitochondrial DNA also led to delayed chondrocyte apoptosis.[11]

Chu et al.[23] emphasized that single dose intra-articular bupivacaine needed more investigation, which led to our pilot study described in chapter 2.

CHAPTER 2

A CASE CONTROLLED EXPERIMENT TO EVALUATE THE IN VIVO EFFECTS OF SINGLE DOSE INTRA-ARTICULAR 0.5% BUPIVACAINE ON CARTILAGE OF SHEEP

2.1 INTRODUCTION

It is difficult to extrapolate data from rats to the human race, because articular biomechanical differences exist between species, and simulation of therapeutic dosages of local anesthetics may be imprecise. The ovine stifle joint has been used increasingly in experimental studies because of its similarities to the human knee. Besides minor differences, such as a smaller trochlear width and a narrower femoral intercondylar notch, the articular surfaces and biomechanics are very similar.[24] According to Osterhoff et al.[24], the ovine stifle joint can be assumed to be approximately a third of the adult human knee in size.

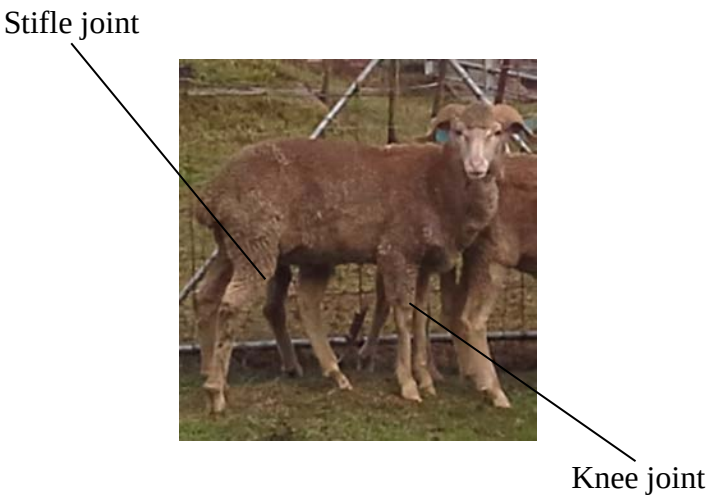

Fig 2.1: Illustration of the stifle and knee joints used

With the question concerning the safety of single dose intra-articular bupivacaine not yet clarified, we conducted a pilot study to simulate a single dose of intra-articular 0.5% bupivacaine on four, 18 month old, merino sheep. The study was a case-controlled experiment and was unpaired, as each sheep was its own control. The nul hypothesis was that a single dose of intra-articular bupivacaine did not cause chondrolysis when compared with intra-articular normal saline. Ethics approval was obtained from the University of Stellenbosch Research Ethics Committee (SU-ACUM13-00004). The project was funded by the Harry Crossley Foundation.

2.2 METHODOLOGY

The animals were fasted for 8 hours prior to the experiment in order to reduce gastro-oesophageal reflux and aspiration risks during the sedation that was given. The sheep were sedated with intra-muscular ketamine 5mg/kg.

In the front legs, the right knee was injected with 7ml 0.5% bupivacaine, while 7ml 0.9% saline was injected into their left knee. This supra-normal dosage was the maximum amount which would fill the joint cavity without increasing intra-articular pressure. The knee cavity of the front leg is considerably smaller than the stifle joint. This dosage addressed the question whether a single intra-articular high dose of bupivacaine can cause significant chondrolysis.

In the hind legs, 7ml 0.5% bupivacaine was given in the right stifle joint, while 7ml 0.9% saline was given in the left stifle joint. This was approximately a third of the volume usually administered to human adult knees. This dosage was comparable to that given to humans in the clinical setting and investigated whether a single, therapeutic dosage causes significant chondrolysis.

The injections were done according to the same sterility protocols used in humans, to reduce the risk of intra-articular sepsis. Their pulse and breathing patterns were monitored until fully awake, and supplemental oxygen was administered throughout the procedure to keep their oxygen saturation above 90%. The animals were observed for the following 5 days for any acute complications of intra-articular injections, such as haemarthrosis and septic arthritis. (See Appendix A for example of monitoring sheet.) After the observation period, the animals were allowed to roam freely in camps on a farm for 6 months. This was to mimic a normal mobile functional human being. When the total of 6 months had expired the animals were taken back to the animal unit for sampling of their joint cartilage. Ketamine 5mg/kg intra-muscularly was given as a premed. Intravenous access was obtained and the animals were given an intravenous dosage of thiopentone 10mg/kg, and a muscle relaxant, pancuronium 0.2mg/kg, followed by a lethal dose of potassium chloride 100mg/kg to serve as cardioplegia. After asystole was sustained and the sheep were declared dead, the knee and stifle joints were collected for histological evaluation of the cartilage.

Cartilage was graded according to the Modified Mankin Score. The parameters used by the Modified Mankin Grading System are surface integrity, cellularity, cell cloning and safranin-O staining intensity (safranin-O binds stoichiometrically to chondroitin 6-sulphate and keratin sulphate in cartilage tissue sections). A higher score implicates more damage to chondrocytes.

These parameters gave each sample a score out of 23 as described below in table 2.1:

Table 2.1: Modified Mankin Score

ARTICULAR SURFACE INTEGRITY	**SCORE**
Normal	0
Slight surface irregularities	1
Moderate surface irregularities	2
Severe surface irregularities	3
Clefts to transitional zone	4
Clefts to radial zone	5
Clefts to calcified zone	6
Fibrillation and/or loss to transitional zone	7
Fibrillation and/or loss to radial zone	8
Fibrillation and/or loss to calcified zone	9
Fibrillation and/or loss to subchondral zone	10
CELLULARITY	
Normal	0
Slight focal decrease	1
Moderate decrease	2
Severe decrease (50% of cells)	3
Complete loss of cells	4
CLONE FORMATION	
None	0
Several doublets	1
Many doublets	2
Doublets and triplets	3
Multiple cell nests	4
SAFRANIN-O STAINING	
Normal	0
Slight reduction	1
Reduction in radial layer	2
Reduction in inter-territorial layer	3
Only present in peri-cellular matrix	4
No staining	5
TOTAL	**23**

In the front knee joints, 5 cartilage biopsies were taken as illustrated in figure 2.2:

1. os carpi ulnare
2. os carpi intermedium
3. os carpi radiale
4. os carpale quartum
5. os carpale tertium

Each biopsy was given a score out of 23, giving each joint a total score from the 5 samples.

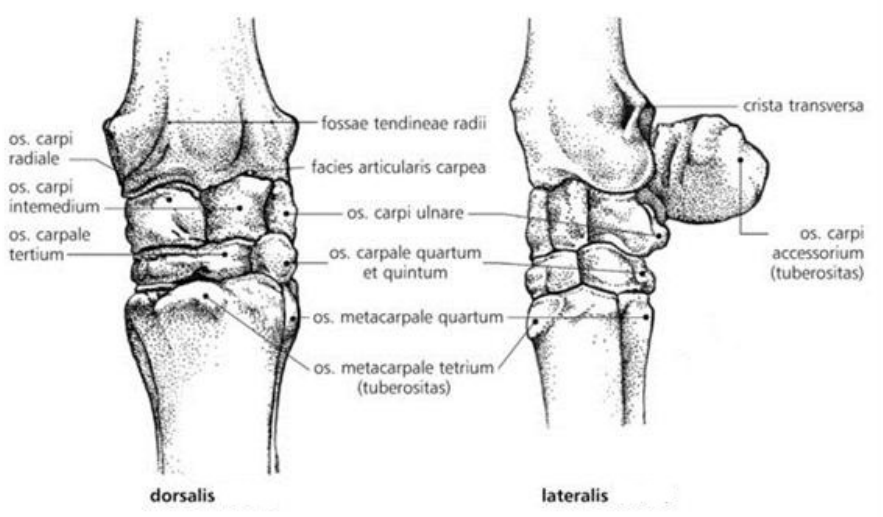

Fig 2.2: Anatomy of the knee of domestic animals[33]

In each stifle joint, 15 cartilage biopsies were taken from 15 predetermined, anatomically distinct positions as illustrated in figure 2.3:

1. anterior patella
2. posterior patella
3. anterior femoral groove
4. central femoral groove
5. posterior femoral groove
6. anterior lateral femoral condyle
7. posterior lateral femoral condyle
8. anterior medial femoral condyle
9. posterior medial femoral condyle
10. anterior lateral tibial plateau
11. posterior lateral tibial plateau
12. central lateral tibial plateau

13. anterior medial tibial plateau
14. posterior medial tibial plateau
15. central medial tibial plateau

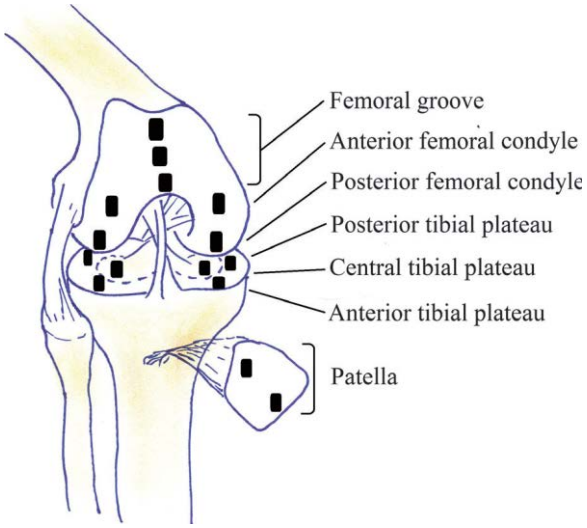

Fig 2.3: Anatomical predetermined positions for sampling of the stifle joint[25]

This sampling method and sites were similar to that used by HR Moody et al.[25] Each stifle joint was then given the total score from the 15 samples.

In order to do the histological analysis, thin sections of the biopsied cartilage were made with a sharp trimming blade. The tissue sections were placed in correctly labeled histology cassettes with appropriate perforations for tissue processing. Processing in graded alcohols and formaldehyde was done using the *Shandion Elliot Duplex* processor overnight for 17 hours. This was followed by orientating the tissue sections within a mould, which was filled with liquid paraffin wax using the *Leica EG 1160* embedder. Once the liquid wax was set and the cassettes were trimmed to expose the tissue, tissue sections of approximately 6µm were cut using a *Leica TM2125RT* microtome. Two sections were cut from one cassette and placed on two different glass slides. The sections were placed in an incubator for approximately 2 hours prior to staining with hematoxylin and eosin (H&E) using a *Leica Autostainer XL*. The safranin-O special stain was performed at a later stage. For this stain, the sections were de-paraffinized and hydrated in different graded alcohols (100%, 95%, and 70%) for one minute each. The sections were subsequently stained with Weigert's iron hematoxylin solution for 10 minutes, followed by a 10 minute washing step using running tap water. Fast green (FCF) solution was used to stain the sections for 5 minutes after which 1% (glacial) acetic acid was used to rinse the sections for a maximum of 15 seconds. The sections were then placed in 0.1% safranin-O solution for 5 minutes before dehydrating the sections in graded alcohols. Cover slips were added using a mounting medium, in this case, DPX (Merck, Germany).

The slides were viewed with an *Axioskop Zeiss* light microscope and microphotographs were obtained using an attached *Zeiss Axiocam* camera.

The right front knees formed the experimental group 1, and were compared to the left front knees, which was our control group 1. The right stifle joints formed experimental group 2, and were compared to the left stifle joints, which was our control group 2. Figure 2.4 is a diagram illustrating the layout of the study:

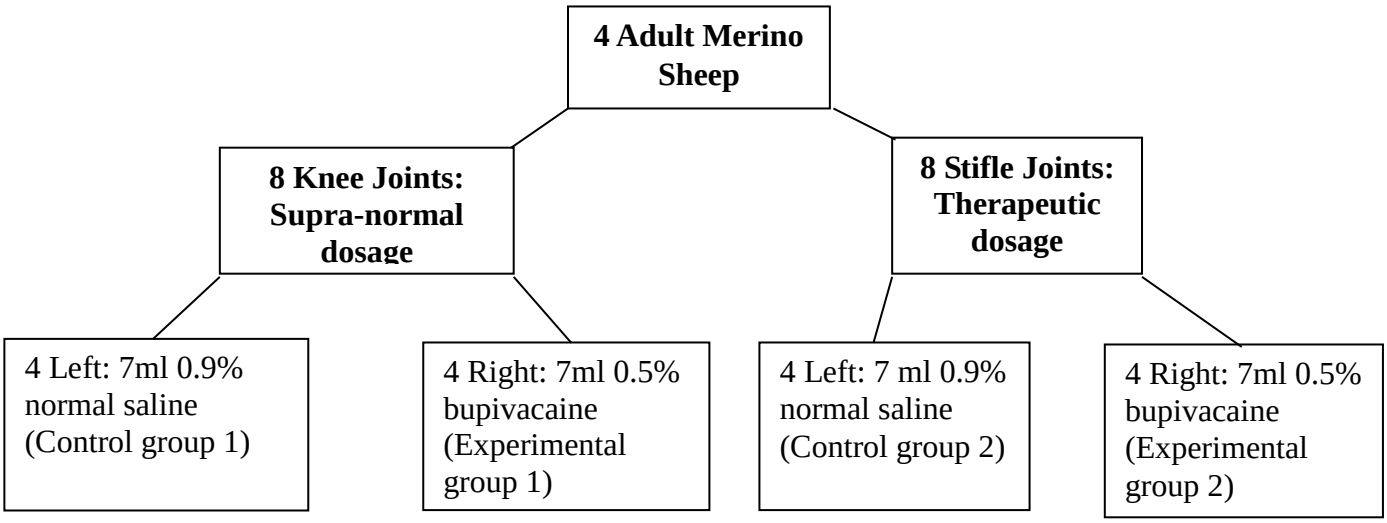

Figure 2.4: Diagram of pilot study

2.3 RESULTS

2.3.1 SUPRA-NORMAL SINGLE DOSE IN KNEE JOINTS

As described above, 5 samples were taken from each knee joint, namely:

1. os carpi ulnare
2. os carpi intermedium
3. os carpi radiale
4. os carpale quartum
5. os carpale tertium.

Each one of these biopsies was graded individually by the Modified Mankin Score. From Sheep 2, the right os carpale quartum sample was insufficient for histological analysis. The contralateral (left) os carpale quartum sample from Sheep 2 was therefore also excluded from the data.

Table 2.2 summarizes the Modified Mankin Scores that was found in the knee joints. (See Appendix

B for full details of the Modified Mankin Scores of the knee joints.) The total scores indicated in table 2.2 are the totals including all 5 samples from each joint. In Sheep 2 it is the total of the remaining 4 valid samples. The average score per knee joint and the average score per biopsy was also calculated.

Table 2.2

Total Score Comparisons	**Right Knee**	**Left Knee**
Sheep 1	21	17
Sheep 2	14	21
Sheep 3	35	16
Sheep 4	17	22
Totals	87	76
Total Average Score per joint	21.75	19
Average Score per biopsy	4,58	4

From the data above, one can see that the total scores of the right joints (bupivacaine group) was slightly more than the left joints (saline group). The data's distribution was not symmetric and Somers' D statistic was therefore used for statistical analysis to account for possible correlation between measurements from the same sheep. The left joints, or the saline group was used as the reference. A positive coefficient indicates higher scores in the right joints (bupivacaine group). Fisher's z transformation was applied on symmetric confidence intervals to obtain asymmetric confidence intervals. Standard errors were obtained using the Jackknife method.[33] Below is the analysis of the Modified Mankin Scores that was found in the knee joints.

Table 2.3

Modified Mankin Score **Knee joints**			
Coefficient	95% Confidence Interval	P Value	Jackknife Standard Error
0.05494505	-0.69603309 to 0.74851313	0.860	0.2873779

There was a 5.49% increase in Modified Mankin Scores for the bupivacaine group. As the 95% confidence interval includes zero, one must statistically assume that there is no statistical difference

between the groups.

Figure 2.5 is a photo taken from a biopsy of a saline injected joint, illustrating normal surface integrity, normal cellularity with only minimal cloning present in the form of doublets:

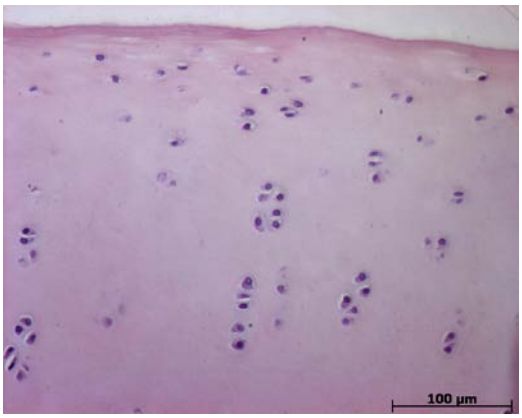

Fig. 2.5: Sheep 3; os carpale tertium, left knee joint

In contrast, figure 2.6 is a biopsy from a bupivacaine injected joint, with severe degenerative changes. Note the surface irregularity, decrease in cellularity and cloning formation in the form of doublets and triplets.

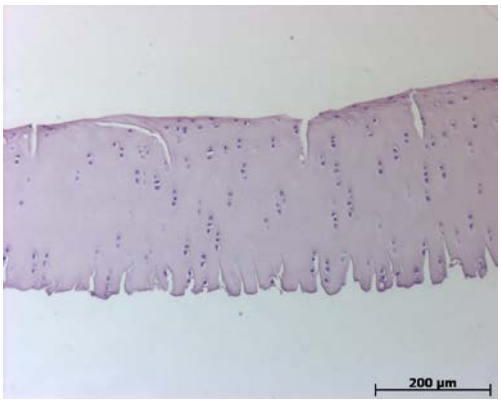

Fig. 2.6: Sheep 3; os carpi ulnare, right knee joint

Differences in safranin-O staining were also observed. Safranin-O normally stains the nuclei black, the cytoplasma blue to green, and the cartilage, mucin and mast cell granules orange to red. Figure 2.7 is an example of normal safranin-O staining:

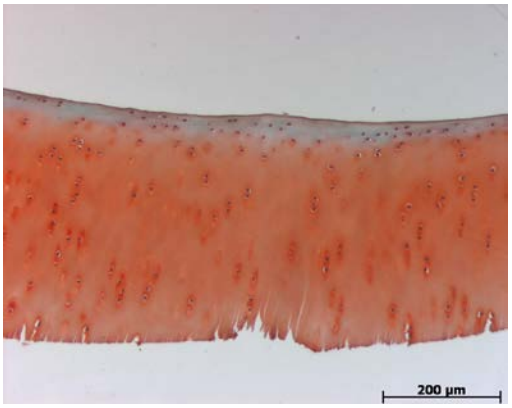

Fig. 2.7: Sheep 3; os carpi ulnare, left knee joint

In contrast, figure 2.8 shows a picture of the contralateral, bupivacaine injected, knee of the same sheep. The safranin-O staining is visibly reduced.

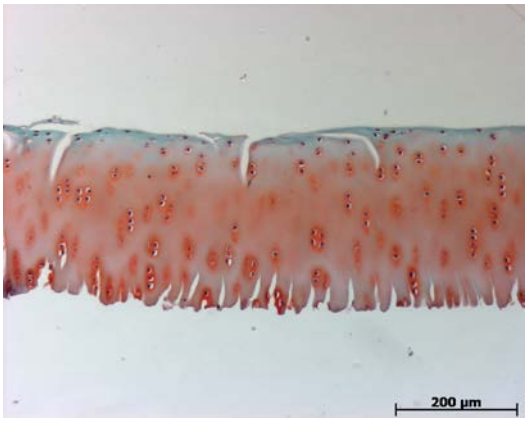

Fig. 2.8: Sheep 3; os carpi ulnare, right knee joint

The different parameters of the Modified Mankin Score were also analysed individually. Tables 2.4, 2.5, 2.6 and 2.7 include the results that were found concerning the articular surface integrity, cellularity, cloning formation and safranin-O staining respectively.

Table 2.4

Articular surface **Knee joints**			
Coefficient	95% Confidence Interval	P Value	Jackknife Standard Error
0.2967033	-0.36444782 to 0.75898096	0.252	0.2161564

Table 2.5

Cellularity **Knee joints**			
Coefficient	95% Confidence Interval	P Value	Jackknife Standard Error
0.10989011	-0.79646316 to 0.86415781	0.789	0.376817

Table 2.6

Clone formation **Knee joints**			
Coefficient	95% Confidence Interval	P Value	Jackknife Standard Error
0.07692308	-0.54032866 to 0.6403521	0.743	0.2142047

Table 2.7

Safranin-O staining **Knee joints**			
Coefficient	95% Confidence Interval	P Value	Jackknife Standard Error
-0.05494505	-0.71882294 to 0.66134718	0.850	0.2671545

From the data above, the greatest difference was seen with articular surface integrity, with an almost 30% reduction in articular surface integrity in the bupivacaine group. The p value was also the lowest at 0.252 for this parameter, but as the 95% confidence interval still includes 0, a significant difference cannot be concluded. Interestingly, analysis of the safranin-O staining resulted in a negative coefficient, indicating a 5% decreased safranin-O staining in the saline group.

2.3.2 THERAPEUTIC SINGLE DOSE IN STIFLE JOINTS

As described in the methodology, 15 samples were taken from each stifle joint, namely;

1. anterior patella
2. posterior patella
3. anterior femoral groove
4. central femoral groove
5. posterior femoral groove
6. anterior lateral femoral condyle
7. posterior lateral femoral condyle
8. anterior medial femoral condyle
9. posterior medial femoral condyle
10. anterior lateral tibial plateau
11. posterior lateral tibial plateau
12. central lateral tibial plateau
13. anterior medial tibial plateau
14. posterior medial tibial plateau
15. central medial tibial plateau

Each one of these biopsies was graded individually by the Modified Mankin Score. From Sheep 1, the biopsy taken from the right posterior lateral tibial plateau was insufficient for histological analysis. The contralateral (left) posterior lateral tibial plateau sample from Sheep 1 was therefore also excluded from the data.

Table 2.8 summarizes the Modified Mankin Scores that were found in the stifle joints. (See Appendix C for full details.) The total scores are the totals including all 15 samples from each joint. In Sheep 1 it is the total of the remaining 14 valid samples. The average scores per joint and sample were also calculated.

Table 2.8

Total Score Comparisons	**Right Stifle joint**	**Left Stifle joint**
Sheep 1	31	33
Sheep 2	57	78
Sheep 3	81	45
Sheep 4	68	65
Totals	237	221
Total Average Score per joint	59.25	55.25
Average Score per biopsy	4.02	3.75

As seen with the supra-normal dosage of intra-articular bupivacaine in the knee joints, we also found higher total Modified Mankin Scores in the bupivacaine group as compared to the normal saline group, with a therapeutic dosage of intra-articular bupivacaine. Somers' D statistic was again used to analyse the Modified Mankin Scores as well as the individual parameters. Tables 2.9 – 2.13 contain the statistical analysis of the data.

Table 2.9

Modified Mankin Score **Stifle joints**			
Coefficient	95% Confidence Interval	P Value	Jackknife Standard Error
0.07233065	-0.49063693 to 0.59269692	0.73	0.1914741

Table 2.10

Articular surface **Stifle joints**			
Coefficient	95% Confidence Interval	P Value	Jackknife Standard Error
-0.08610792	-0.57329343 to 0.44606062	0.661	0.1778799

Table 2.11

Cellularity **Stifle joints**			
Coefficient	95% Confidence Interval	P Value	Jackknife Standard Error
-.01722158	-0.32036783 to 0.28912427	0.873	0.0989282

Table 2.12

Clone formation **Stifle joints**			
Coefficient	95% Confidence Interval	P Value	Jackknife Standard Error
0.03329506	-0.30646364 to 0.36552878	0.782	0.1099611

Table 2.13

Safranin-O staining **Stifle joints**			
Coefficient	95% Confidence Interval	P Value	Jackknife Standard Error
0.22273249	-0.44372443 to 0.73055549	0.381	0.2210212

An increase of 7% in the Modified Mankin Score was found in the bupivacaine injected right stifle joints, as compared to the saline injected left stifle joints. Figure 2.9 shows an example of a biopsy with normal surface integrity, cellularity and no cloning formation taken from a saline injected joint.

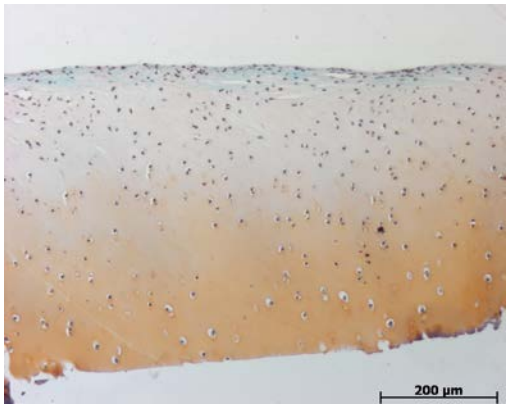

Fig. 2.9: Sheep 1; central lateral tibial plateau, left stifle joint

To illustrate the differences observed in the parameters of the Modified Mankin Score, figure 2.10 from a bupivacaine injected right stifle joint, shows an irregular articular surface with abnormal cellularity and clone formation in the form of doublets.

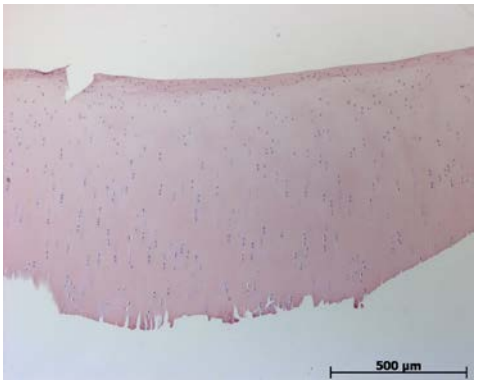

Fig. 2.10: Sheep 2; central medial tibial plateau, right stifle joint

These degenerative changes were not only limited to our bupivacaine group. The sample illustrated in figure 2.11 below, was taken from the left stifle joint in sheep 2 (saline group) and shows severe surface irregularities, cloning formation in the form of doublets and focal decrease in cellularity.

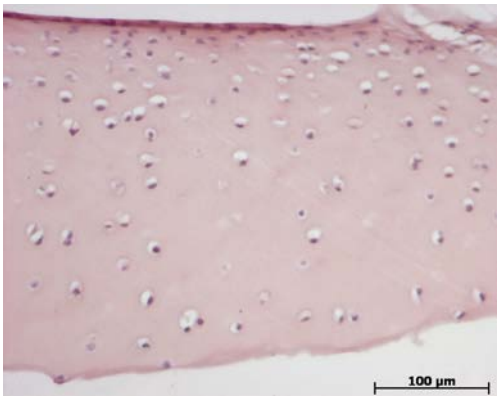

Fig. 2.11: Sheep 2; posterior lateral tibial plateau, left stifle joint

From the individual parameters assessed, the biggest difference between the bupivacaine injected joints and the saline control joints, was found in safranin-O staining, with a 22% reduction in staining in the bupivacaine group ($p = 0.381$). Figure 2.12 is an example of normal safranin-O staining from the control, left stifle joint.

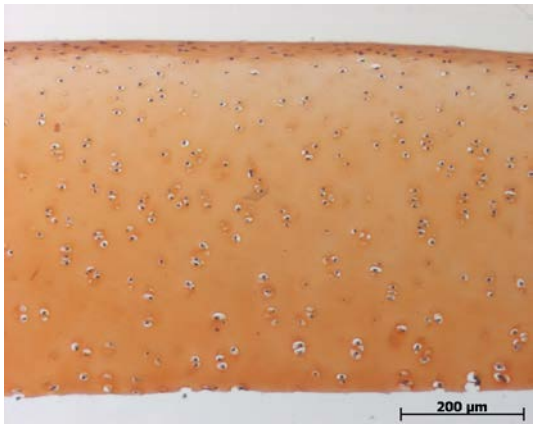

Fig. 2.12: Sheep 1; anterior lateral femoral condyle, left stifle joint

Complete loss of safranin-O staining was found in a bupivacaine injected right stifle joint, as shown in figure 2.13.

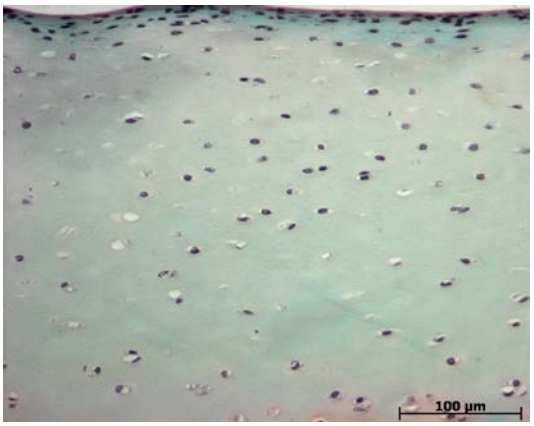

Fig. 2.13: Sheep 3; posterior lateral tibial plateau, right stifle joint

CHAPTER 3
DISCUSSION

3.1 LITERATURE REVIEW

Numerous in vitro and in vivo studies confirmed the chondrotoxic effect of bupivacaine. Ropivacaine may be the least toxic local anesthetic agent for intra-articular use.[18] One in vivo study suggested that bupivacaine's enantiomer, levobupivacaine, may be safe.[20] In this study the time from intra-articular levobupivacaine administration and sampling of the cartilage for histological analysis was only 21 days, which could falsely increase the likelihood of negative results as changes become more prevalent at longer intervals after administration.[11] One in vitro study even found levobupivacaine to be more toxic to chondrocytes than bupivacaine.[19] The chondrotoxic effect of bupivacaine is a uniform finding over numerous species, as investigated in dogs, rats and humans.[13-15]

The addition of epinephrine to intra-articular bupivacaine did not increase the risk of chondrotoxicity in an in vivo rat model.[12] In contrast, in an in vitro study on human chondrocytes, the addition of epinephrine did cause more chondrolysis.[13] Cartilage cells in joint cavities have a very limited blood supply. Epinephrine is a very strong vasoconstrictor. This should caution against its use as additive to intra-articular bupivacaine.

The duration of exposure of chondrocytes to bupivacaine has proved to increase cartilage damage.[14] One in vitro study suggested that an infusion time of less than 48 hours may be safe.[13] Chondrotoxicity due to bupivacaine is also dependent on the intra-articular dosage used.[16] The total dosage of bupivacaine is a product of the concentration and volume used. By reducing either the volume or the concentration, one can therefore limit the total dosage administered.

3.2 SINGLE DOSE INTRA-ARTICULAR BUPIVACAINE PILOT STUDY

Total Modified Mankin Scores were calculated in both the knee and stifle joints, after administration of the supra-normal and therapeutic dosages of 0.5% bupivacaine respectively. These were compared to 0.9% saline administration in the contralateral joints, which served as controls. With both dosages, the total scores were higher in the bupivacaine group as compared to the saline group. Statistical analysis of the different parameters, namely, articular surface integrity, cloning formation, cellularity and safranin-O staining, also proved to be higher in the bupivacaine groups,

except for the safranin-O staining in the knee joints (-5,5%). Articular surface integrity (-8.6%) and cellularity (-1.7%) in the stifle joints were also higher in the saline groups.

Statistical analysis could not conclude a significant difference between the groups, as the 95% confidence intervals always included 0. This was only a pilot study, with a very small sample size, limited by the costs involved. A power analysis of the pilot study is also not applicable. It is therefore difficult to draw conclusions with the limited data available. Bigger sample sizes will narrow the 95% confidence interval and improve interpretation.

If we are to assume that equal anatomical samples from different legs are uncorrelated, according to Wilcoxon signed-rank test, there was a significant difference between the bupivacaine and saline groups in safranin-O staining ($p=0.014$) as illustrated in table 3.1. Higher scores were observed in the right stifle joints (bupivacaine group) when compared to the left stifle joints (saline group). There were no differences found in the front knee joints as mentioned above, concerning the safranin-O staining.

Table 3.1

Wilcoxin signed-rank test **Safranin-O Staining: Stifle joints**	
Sign	Observed
Positive	28
Negative	13
Zero	18
Total	59

We therefore conclude that single dose intra-articular bupivacaine may be chondrotoxic, but we need a bigger sample size to confirm our strong suspicion.

3.3 PATHOPHYSIOLOGY OF BUPIVACAINE CHONDROTOXCICITY

The exact mechanism of bupivacaine chondrotoxicity is unclear. A proposed mechanism is disruption of the cell membrane that might cause acute necrosis. This phenomenon increases with increased fat solubility, which is a known characteristic of bupivacaine. Mitochondrial function may also be affected, by disrupting the mitochondrial transmembrane potential, leading to less ATP

production. Alterations in mitochondrial DNA then lead to chondrocyte apoptosis.[11] The agent must diffuse through the intercellular matrix to exert its chondrotoxic effect. Disruption of the superficial layer of cartilage, by example inserting anchor sutures by the surgeon, can increase the risk of chondrotoxicity by damaging the protective barrier of chondrocytes. An intra-articular injectate can then infiltrate the intercellular proteoglycan matrix of the cartilage, with less resistance, and cause its toxic effects to chondrocytes.

Cartilage, compared to other tissues, has limited ability to heal and regenerate itself. Once an insult has occurred, a long term effect becomes possible. Loss of functional cells will result in loss of extracellular matrix and collagen production, which plays a vital role in the integrity of the cartilage tissue.[17]

Because of uncertainty concerning the mechanism of action of bupivacaine chondrotoxicity, can one safely assume cause and effect? In a meta-analysis by Matsen, et al.[11], it was shown that there is indeed "*strong association, consistency, specificity, temporal relationship, biological gradient and plausibility, overall coherence of evidence, good experimental evidence and analogy*". Matsen therefore concluded that bupivacaine does cause chondrocyte damage.[11]

3.4 ALTERNATIVES TO INTRA-ARTICULAR BUPIVACAINE

All local anesthetics have been implicated in chondrocyte death. Ropivacaine seems to be the least toxic.[18] Recently, magnesium, a N-methyl-D-aspartate (NMDA) receptor antagonist, has been used as part of a multimodal analgesic regime for joint pain. NMDA receptors have been found on intra-articular surfaces and Bondok et al.[26] showed that intra-articular magnesium is an effective alternative to intra-articular bupivacaine.[26] Magnesium not only has analgesic properties but also has chondro-proliferative properties.[27] Interestingly, Baker et al.[16] found that 10% magnesium attenuated the chondrotoxic effects of local anesthetics.[16]

Intra-articular steroids have proven to increase the risk of septic arthritis and may even have a synergistic toxic effect on chondrocytes when combined with local anesthetics.[3, 28]

The data on the use of intra-articular morphine has been conflicting. In a qualitative review of 46 randomized controlled trials done by Rosseland et al.[29] in 2005, the conclusion was that intra-articular morphine was an ineffective analgesic for joint surgery.[29] Previous studies used small dosages (example 1 mg) of intra-articular morphine, so Garcia et al.[30] undertook a randomized

controlled trial, in 2010, on 50 patients comparing 10 mg intra-articular morphine with intra-articular normal saline. A significant reduction in rescue analgesia was observed in the morphine group ($p = 0.0001$). The time to request additional analgesia was also longer in the morphine group ($p = 0.0166$). Post-operative pain scores were also reduced at 2 and 6 hours in the morphine group.[30]

Intra-articular alpha 2 agonists such as clonidine have also been used with good effect, but more data is needed for safe conclusions.[3]

CHAPTER 4
CONCLUSION

Intra-articular bupivacaine is common practice in joint analgesia. It's efficacy as analgesic shows good response, but the risk involved is undoubtedly irreversible, and potentially disabling, chondrolysis. Bupivacaine's intra-articular chondrotoxic effect is time, concentration and dose dependent. Using intra-articular bupivacaine infusions increases the risk of chondrocyte toxicity. Single dose intra-articular bupivacaine may also be significantly chondrotoxic. Chu et al.[23] showed a statistically significant 50% reduction in chondrocyte density after a single dose of intra-articular bupivacaine in rats. From our pilot study done on 4 sheep, the overall impression was increased chondrotoxicity in the bupivacaine groups, but statistical significance could not be proven due to a small sample size.

The addition of intra-articular magnesium sulphate appears to attenuate the chondrotoxic effect of local anesthetics and magnesium has a proliferative effect on chondrocytes. High dose intra-articular morphine and intra-articular clonidine may prove beneficial as analgesics for joint surgery, but more data is needed. Poor integrity of the articular surface increases the risk of chondrocyte death from intra-articular medications, which opens the discussion of optimal surgical technique.

APPENDICES

1. **APPENDIX A: Monitoring sheet for sheep wellbeing**

A case controlled experiment to evaluate the in vivo effects of single dose intra-articular 0.5% bupivacaine on cartilage of sheep, 2014

MONITORING SHEET

Date :

Sheep number (1-4) :

Physical Indicators

Acute complications

	Normal	Swelling	Effusion	Induration	Tenderness
Right knee joint					
Left knee joint					
Right stifle joint					
Left stifle joint					

Chronic complications

	Normal	Antalgic
Gait		

Psycological indicators

	Normal	Refusal to eat
Appetite		

Reviewed by: ....

Signature

2. APPENDIX B: Modified Mankin Scores of the Front Knees

	Sample	Side	Articular surface integrity	Cellularity	Clone formation	Safranin-O Staining	Total
Sheep 1 : Front Knee	Os Carpi Ulnare	Right	1	1	1	2	5
	Os carpi intermedium	Right	1	1	1	2	5
	Os Carpi Radiale	Right	1	1	0	1	3
	Os Carpale quartum	Right	2	1	1	1	5
	Os Carpale tertium	Right	0	1	1	1	3
						Total	21
	Os Carpi Ulnare	Left	0	1	3	1	5
	Os carpi intermedium	Left	0	1	3	0	4
	Os Carpi Radiale	Left	0	1	1	1	3
	Os Carpale quartum	Left	1	1	0	3	5
	Os Carpale tertium	Left	0	0	0	0	0
						Total	**17**
Sheep 2 : Front Knee	Os Carpi Ulnare	Right	3	1	1	0	5
	Os carpi intermedium	Right	0	1	1	1	3
	Os Carpi Radiale	Right	1	1	1	0	3
	Os Carpale quartum	Right		Sample not suitable for analysis			
	Os Carpale tertium	Right	1	1	1	0	3
						Total	14
	Os Carpi Ulnare	Left	3	1	2	1	7
	Os carpi intermedium	Left	0	1	2	2	5
	Os Carpi Radiale	Left	3	1	1	0	5
	Os Carpale quartum	Left					
	Os Carpale tertium	Left	2	0	1	1	4
						Total	**21**
Sheep 3 : Front Knee	Os Carpi Ulnare	Right	4	2	2	2	10
	Os carpi intermedium	Right	3	1	2	4	10
	Os Carpi Radiale	Right	0	1	0	2	3
	Os Carpale quartum	Right	2	1	2	2	7
	Os Carpale tertium	Right	1	1	2	1	5
						Total	35
	Os Carpi Ulnare	Left	0	0	1	1	2
	Os carpi intermedium	Left	1	0	1	2	4
	Os Carpi Radiale	Left	2	0	1	2	5
	Os Carpale quartum	Left	0	1	1	2	4
	Os Carpale tertium	Left	0	0	1	0	1
						Total	**16**
Sheep 4 : Front Knee	Os Carpi Ulnare	Right	3	0	1	1	5
	Os carpi intermedium	Right	0	0	0	0	0
	Os Carpi Radiale	Right	0	0	0	0	0
	Os Carpale quartum	Right	0	0	3	2	5
	Os Carpale tertium	Right	3	0	3	1	7
						Total	17
	Os Carpi Ulnare	Left	2	1	1	1	5
	Os carpi intermedium	Left	0	0	0	2	2
	Os Carpi Radiale	Left	0	1	3	2	6
	Os Carpale quartum	Left	0	1	0	1	2
	Os Carpale tertium	Left	3	1	1	2	7
						Total	**22**

3. APPENDIX C: Modified Mankin Scores of the Stifle joints

	Sample	Side	Articular surface integrity	Cellularity	Clone formation	Safranin-O Staining	Total
Sheep 1: Stifle joint	Ant Patella	Right	1	0	1	0	2
	Post Patella	Right	1	1	0	0	2
	Ant Femoral Grove	Right	0	0	1	1	2
	Central Femoral Grove	Right	1	0	0	1	2
	Post Fem Grove	Right	1	1	0	1	3
	Ant Lat Fem Grove	Right	0	0	0	1	1
	Post Lat Fem Condyle	Right	0	0	0	0	0
	Ant Med Fem Condyle	Right	0	0	0	0	0
	Post Med Fem Condyle	Right	1	0	0	2	3
	Ant Lat Tib Plateau	Right	1	0	0	2	3
	Post Lat Tib Plateau	Right		Sample not suitable for analysis			
	Cent Lat Tib Plateau	Right	0	0	0	1	1
	Ant Med Tib Plateau	Right	1	1	0	1	3
	Post Med Tib Plateau	Right	1	1	0	0	2
	Cent Tib Plateau	Right	2	1	1	3	7
						Total	31
	Ant Patella	Left	0	1	1	1	3
	Post Patella	Left	1	0	0	0	1
	Ant Femoral Grove	Left	0	0	0	0	0
	Central Femoral Grove	Left	1	1	1	1	4
	Post Fem Grove	Left	0	1	2	2	5
	Ant Lat Fem Grove	Left	0	0	0	0	0
	Post Lat Fem Condyle	Left	0	0	0	3	3
	Ant Med Fem Condyle	Left	1	1	1	1	4
	Post Med Fem Condyle	Left	0	0	0	0	0
	Ant Lat Tib Plateau	Left	0	0	1	0	1
	Post Lat Tib Plateau	Left					
	Cent Lat Tib Plateau	Left	0	0	0	3	3
	Ant Med Tib Plateau	Left	0	1	0	1	2
	Post Med Tib Plateau	Left	0	0	1	3	4
	Cent Tib Plateau	Left	0	0	0	2	2
						Total	**32**

	Sample	Side	Articular surface integrity	Cellularity	Clone formation	Safranin-O Staining	Total
Sheep 2: Stifle joint	Ant Patella	Right	0	0	0	1	1
	Post Patella	Right	1	0	0	2	3
	Ant Femoral Grove	Right	2	0	1	0	3
	Central Femoral Grove	Right	0	1	3	1	5
	Post Fem Grove	Right	0	0	0	1	1
	Ant Lat Fem Grove	Right	1	0	0	1	2
	Post Lat Fem Condyle	Right	0	0	0	0	0
	Ant Med Fem Condyle	Right	1	1	1	2	5
	Post Med Fem Condyle	Right	0	0	0	2	2
	Ant Lat Tib Plateau	Right	1	1	1	3	6
	Post Lat Tib Plateau	Right	3	1	1	5	10
	Cent Lat Tib Plateau	Right	0	1	1	0	2
	Ant Med Tib Plateau	Right	0	1	0	2	3
	Post Med Tib Plateau	Right	3	1	1	0	5
	Cent Tib Plateau	Right	4	1	2	2	9
						Total	57
	Ant Patella	Left	0	1	0	2	3
	Post Patella	Left	3	1	1	2	7
	Ant Femoral Grove	Left	1	1	1	0	3
	Central Femoral Grove	Left	1	1	3	1	6
	Post Fem Grove	Left	0	1	0	1	2
	Ant Lat Fem Grove	Left	2	1	1	5	9
	Post Lat Fem Condyle	Left	0	0	0	0	0
	Ant Med Fem Condyle	Left	2	0	0	1	3
	Post Med Fem Condyle	Left	4	1	0	3	8
	Ant Lat Tib Plateau	Left	0	0	0	0	0
	Post Lat Tib Plateau	Left	3	1	2	5	11
	Cent Lat Tib Plateau	Left	3	1	1	2	7
	Ant Med Tib Plateau	Left	2	1	1	2	6
	Post Med Tib Plateau	Left	2	1	3	1	7
	Cent Tib Plateau	Left	3	1	0	3	7
						Total	**79**

	Sample	Side	Articular surface integrity	Cellularity	Clone formation	Safranin-O Staining	Total
Sheep 3: Stifle joint	Ant Patella	Right	1	0	0	3	4
	Post Patella	Right	0	1	3	3	7
	Ant Femoral Grove	Right	0	1	3	2	6
	Central Femoral Grove	Right	0	0	1	5	6
	Post Fem Grove	Right	0	0	0	4	4
	Ant Lat Fem Grove	Right	0	0	1	2	3
	Post Lat Fem Condyle	Right	1	0	2	3	6
	Ant Med Fem Condyle	Right	2	0	1	2	5
	Post Med Fem Condyle	Right	2	1	0	2	5
	Ant Lat Tib Plateau	Right	2	1	1	3	7
	Post Lat Tib Plateau	Right	2	1	0	5	8
	Cent Lat Tib Plateau	Right	2	0	0	5	7
	Ant Med Tib Plateau	Right	1	0	1	1	3
	Post Med Tib Plateau	Right	2	1	1	5	9
	Cent Tib Plateau	Right	0	0	0	1	1
						Total	81
	Ant Patella	Left	0	0	0	1	1
	Post Patella	Left	0	0	0	1	1
	Ant Femoral Grove	Left	1	1	2	2	6
	Central Femoral Grove	Left	1	0	3	1	5
	Post Fem Grove	Left	0	0	0	2	2
	Ant Lat Fem Grove	Left	3	0	0	0	3
	Post Lat Fem Condyle	Left	3	1	0	2	6
	Ant Med Fem Condyle	Left	1	0	0	1	2
	Post Med Fem Condyle	Left	2	0	0	1	3
	Ant Lat Tib Plateau	Left	1	1	0	0	2
	Post Lat Tib Plateau	Left	1	1	0	5	7
	Cent Lat Tib Plateau	Left	0	0	1	0	1
	Ant Med Tib Plateau	Left	1	0	0	0	1
	Post Med Tib Plateau	Left	0	1	1	0	2
	Cent Tib Plateau	Left	3	1	0	0	4
						Total	**46**

	Sample	Side	Articular surface integrity	Cellularity	Clone formation	Safranin-O Staining	Total
Sheep 4: Stifle joint	Ant Patella	Right	1	0	0	5	6
	Post Patella	Right	0	0	3	1	4
	Ant Femoral Grove	Right	0	1	1	1	3
	Central Femoral Grove	Right	0	1	1	2	4
	Post Fem Grove	Right	1	0	0	2	3
	Ant Lat Fem Grove	Right	2	1	1	2	6
	Post Lat Fem Condyle	Right	0	1	1	0	2
	Ant Med Fem Condyle	Right	3	1	3	0	7
	Post Med Fem Condyle	Right	1	1	1	1	4
	Ant Lat Tib Plateau	Right	1	1	0	2	4
	Post Lat Tib Plateau	Right	3	2	0	2	7
	Cent Lat Tib Plateau	Right	0	2	0	2	4
	Ant Med Tib Plateau	Right	1	1	0	2	4
	Post Med Tib Plateau	Right	1	1	0	5	7
	Cent Tib Plateau	Right	0	3	0	0	3
						Total	68
	Ant Patella	Left	1	0	1	2	4
	Post Patella	Left	3	1	0	0	4
	Ant Femoral Grove	Left	1	1	3	0	5
	Central Femoral Grove	Left	3	0	0	1	4
	Post Fem Grove	Left	0	0	1	0	1
	Ant Lat Fem Grove	Left	1	0	0	1	2
	Post Lat Fem Condyle	Left	2	0	0	0	2
	Ant Med Fem Condyle	Left	1	0	0	0	1
	Post Med Fem Condyle	Left	1	1	3	2	7
	Ant Lat Tib Plateau	Left	3	1	0	0	4
	Post Lat Tib Plateau	Left	5	3	0	2	10
	Cent Lat Tib Plateau	Left	0	0	1	2	3
	Ant Med Tib Plateau	Left	1	2	0	2	5
	Post Med Tib Plateau	Left	5	3	0	1	9
	Cent Tib Plateau	Left	2	1	1	0	4
						Total	**65**

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